

# Metabolite-based mutualism between *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* enhances current generation in bioelectrochemical systems

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## Abstract

Understanding the ecological relationships of the microbiota in bioelectrochemical systems (BESs) is necessary to gain deeper insight into their performance. Here, we show that the fermentation product 2,3-butanediol stimulates mutually beneficial interactions between *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* in a BES with glucose as the initial substrate under microaerobic conditions. The experiments were conducted in potentiostatically poised 3-electrode reactors. Under these conditions: (i) the current density by a co-culture of *P. aeruginosa* and *E. aerogenes* increased at least 14-fold compared to the current density by either of these two bacteria alone; and (ii) *E. aerogenes* fermented glucose principally to 2,3-butanediol, which was subsequently consumed by *P. aeruginosa*. To determine the benefits to each microorganism in this symbiosis, we conducted experiments with pure cultures. The current production by a pure culture of *P. aeruginosa* with 2,3-butanediol was increased 2-fold compared with glucose as the carbon source. This was due to enhanced phenazine production by *P. aeruginosa*. Further, pyocyanin comprised the majority (92%) of the phenazines produced by *P. aeruginosa* with

2,3-butanediol, but only 29% with glucose. The current production by a pure culture of *E. aerogenes* increased ~19-fold when the growth medium was supplemented with 35  $\mu\text{g ml}^{-1}$  of pyocyanin as the electron mediator. We also observed that *E. aerogenes* generated maximum current densities with pyocyanin compared to the other three phenazines, indicating that *E. aerogenes* respire most effectively with pyocyanin—the phenazine which production is stimulated by this microbe's product (2,3-butanediol). Concomitantly, a decrease in fermentation products and enhanced growth with increasing concentrations of pyocyanin implies a shift towards electrode-based respiration by *E. aerogenes* rather than fermentation. Therefore, the synergism in current generation by the co-culture can be attributed to the combination of enhanced pyocyanin production by *P. aeruginosa* with 2,3-butanediol and the ability of *E. aerogenes* to efficiently respire. This study is the first to demonstrate metabolite based “inter-species communication” in BESs, resulting in enhanced electrochemical activity. It also explains how an inconsequential fermenter can become an important electrode-respiring bacterium within an ecological network at the anode.

## Involved units

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## Identifier

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